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REVIEW

Stem cells: potency, plasticity and public perception*

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Abstract

Production of chimaeras with embryonal carcinoma and embryonic stem cells enabled a very thorough investigation of the potency of these cells in the mouse. Human embryonal carcinoma and embryonic stem cell differ from their murine counterparts in a number of respects and, for obvious reasons, their potency is more difficult to assess. Recently, findings attesting to a surprising degree of plasticity of cells from adults have begun to emerge, which, aside from offering a possible further route to stem cell therapy, raise intriguing questions about the importance of lineage in the process of cellular diversification. Biomedical research is widely perceived to be advancing too fast to allow proper consideration of the implications of its clinical applications. Whilst this was clearly not true in the case of human *in vitro* fertilization, it has some validity regarding stem cell therapy, even though many of the issues are common to both. Casual use of the term 'embryo' proved unhelpful in the past debate on whether research on early stages of human development should be permitted. Likewise, introduction of the term 'therapeutic cloning' has complicated the present one regarding extension of such research to stem cell therapy.

Key words cell lineage; embryonal carcinoma cell; embryonic stem cells; theraputic cloning.

Introduction

Stem cell research has reached the exciting stage of offering the prospect of restoring normal function to a much wider variety of tissues damaged by serious disease or injury than could have been contemplated just a few years ago. However, many issues need to be addressed before such aspirations can be fully realized. These include the most suitable source of starting cells, how to obtain pure populations of the desired types of differentiated cells, and the extent to which the latter need to be organized and retain mitotic potential in order to yield effective grafts. There is the further important issue of how the problem of graft rejection can best be circumvented. These are only a few of the scientific issues confronting those seeking to exploit stem cells therapeutically. However, additional considerations that cannot be ignored include access to material. While human assisted reproduction has provided abundant material for deriving human embryonic stem (ES) cells, whether it should be used for this purpose is a matter on which opinion remains sharply divided. For most types of adult stem cells, recently deceased donors would be required. Within the United Kingdom not only is there already an acute shortage of such donors for organ transplantation, but consent for removal of brain tissue, for example, may prove difficult to obtain. Use of tissue from aborted fetuses, though permissible in the UK, also remains contentious. Hence, an attractive alternative is to use extra-embryonic tissues that are discarded at birth, among which stem cells in cord blood are an obvious choice. However, enriching for such cells and securing their proliferation in culture are necessary before their therapeutic potential can be evaluated.

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Pluripotency

For many years, embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas derived from germ cells or from ectopically grafted embryos in mice, were exploited intensively as an *in vitro* system for studying aspects of differentiation that normally take place during the

relatively inaccessible early post-implantation stages of embryogenesis (e.g. Hogan et al. 1983). Evidence that EC cells were pluripotent first came from heroic experiments entailing more than 1700 grafts of single core cells from simple embryoid bodies (EBs) to syngeneic adults mice (Kleinsmith & Pierce, 1964). Forty-four of the hosts developed tumours, all but one of which showed the same range of distinct types of differentiation as the parent tumour from which the donor cell was derived. Subsequently, the conclusion that EC cells were truly pluripotent was confirmed and extended by studies in which single such cells were injected into blastocysts (Illmensee & Mintz, 1976). Here, progeny of the single transplanted EC cells were found to be able to contribute substantially to all somatic tissues that were analysed in the resulting chimaeras, and to do so in most cases without also producing tumours. Hence when placed in this more appropriate embryonic environment, formation of tumours by these cells was the exception rather than the rule. Clearly, EC cells can exhibit pluripotency in widely differing circumstances, but their retention of the capacity to self-renew is more circumscribed.

Particularly when encouraged to form simple and then cystic EBs, EC cells can also differentiate *in vitro* into cells representative of derivatives of all three primary germ layers (reviewed in Gardner, 1983). The fact that their repertoire was more limited in these circumstance than *in vivo* is not surprising in view of the dependence of differentiation of many types of cells on interactions between dissimilar tissues. What carried interest in EC cells beyond a simple *in vitro* system for studying differentiation was the claim that, in addition to contributing to somatic tissues following injection into blastocysts, they could also colonize the germline (Mintz & Illmensee, 1975; Stewart & Mintz, 1981). This was notable because it offered the exciting prospect of genetically modifying prospective germ cells under *in vitro* conditions where rare events could be both orchestrated and selected for. However, a contribution by EC cells to the germline proved exceptional even in the single laboratory for which success was reported. Nevertheless, it provided the vital impetus for stimulating efforts to obtain better EC cells by using direct explantation of early conceptuses to circumvent the very lengthy process by which they had hitherto been derived *in vivo*. It was this that eventually led to the production of embryonic stem (ES) cells (Evans & Kaufman, 1981; Martin, 1981). Whether or not these cells can be regarded strictly as euploid EC cells, they behave very similarly to them both in ectopic grafts to syngeneic adults and following blastocyst injection. The only respects in which ES cells differ obviously from EC cells is that they can contribute routinely to the germline (Bradley et al. 1984), and form tumours very rarely when injected into blastocysts as opposed to adult hosts.

EC lines derived from tumours of germ cell origin have long served as the human counterpart of murine EC cells for studying aspects of human development and differentiation *in vitro* (reviewed in Andrews et al. 2001). However, human EC cells differ sufficiently in morphology and pattern of growth from those of the mouse as to raise doubts about whether they indeed correspond in developmental status. Recent success in deriving ES and EG lines directly from human conceptuses confirm that these more closely resemble human rather than murine EC or ES cells in all respects (Shamblott et al. 1998; Thomson et al. 1998). That human EC and ES cells display stem cell characteristics is clear from the various markers they have been found to express (Andrews et al. 2001). The question of whether they rival their murine counterparts in potency is harder to address because the critical assays employed to demonstrate this for the mouse, namely blastocyst injection and ectopic grafting in adult hosts, are obviously not options in our species. This leaves xenogeneic grafting and induction of differentiation *in vitro*. What both these assays reveal is that human EC and ES cells can differentiate into a range of different cell types. Moreover, clonally derived human ES cell lines have been shown to be able to produce differentiated derivatives characteristic of all three primary germ layers (Amit et al. 2000). Hence, within the limits of permissible assays, there is a reasonable case for accepting that the cells in question are the human counterpart of mouse ES cells. However, it is notewothy in this context that while there is no compelling evidence that murine ES cells include trophoblast in their repertoire of differentiation (Brook & Gardner, 1997), human EC and ES cell evidently do so routinely (Andrews et al. 2001). This raises the interesting possibility that the production of ES cells in the human entails uncoupling of proliferation from further differentiation at an earlier stage of ontogeny than in the mouse. Nevertheless, the mouse is still the only mammal from which ES cells that can form functional gametes have been obtained, and even within this species only some strains have yielded positive results. So far as mammals other than mouse

and man are concerned, attempts to derive 'ES-like' cells have met with rather variable success (reviewed in Gardner & Brook, 1997).

There is now the prospect, the first steps towards the realization of which have already been taken, of using human ES cells to obtain specialized types of cell *in vitro* for repairing tissues damaged by disease or injury (see contributions by **Mummery** and by **Itskovitz**). At present, a problem that bedevils this approach to stem cell therapy is that, even in the mouse, the best that can be achieved is a strong bias towards a particular type of differentiation. This means that substantial numbers of cells of other, usually undefined, types may be present that could impair the efficacy of grafts. One approach to the elimination of inappropriate cells is to transfect ES cells with constructs in which the coding region of an antibiotic resistance gene is coupled to the promoter of a gene that is expressed in the desired cell type. This means that only cells expressing the gene in question will survive exposure to the antibiotic (Klug et al. 1996; Li et al. 1998). There are, however, two obvious potential problems with this strategy. First, it is based on the premise, which may prove difficult to validate, that only the desired type of differentiated cell expresses the chosen gene at a sufficient level to confer antibiotic resistance. Second, in an era of general public disquiet about genetic modification, such a strategy is very unlikely to be acceptable in a clinical as opposed to laboratory context. Does it really matter if a graft is not composed wholly of the desired type of cell? In seeking to answer this, two points are worth noting. The first is that the long-term consequences of grafting one type of specialized cell into an environment appropriate for another is not known. The second concerns inclusion of persisting undifferentiated ES cells in grafts. In the work of Kleinsmith & Pierce (1964) cited earlier, the incidence of tumours among peritoneal grafts of single cells from simple EBs was 11%. Especially since the starting material was not a pure population of EC cells, this is a dramatic testimony to the tumorigenic potential of these cells and hence, by implication, of ES cells. This work underscores the need in a therapeutic context to ensure that differentiated cultures are purged of all ES cells before contemplating their grafting.

Plasticity and the problem of lineage

The idea of using ES cell for therapeutic purposes had hardly gained general acceptance within the biomedical research community than the option of using cells from adults as an alternative started to gain credibility. There is now a wealth of literature suggesting that cells from a variety of adult tissues retain much wider options for differentiation than had been assumed hitherto (Morrison, 2000). In most cases the work is still at the stage of intriguing phenomenology rather than incisive analysis, so its significance remains difficult to assess (Anderson et al. 2001). It does, however, raise intriguing questions about the role of cell lineage in development and differentiation.

It is evident that the existence of more or less stereotyped lineages is not attributable to there being only a single route to a final type of differentiated cell, i.e. that the genome must undergo a defined sequence of epigenetic changes in order to attain a specific set of differentiated states. This is demonstrated most graphically in the nematode, *Caenorhabditis elegans*, an organism whose essentially invariant cell lineage has been fully charted from the zygote through to adulthood (Sulston & Horvitz, 1977). Here it is clear that specific types of differentiated cell arise in a variety of different lineage contexts. In vertebrates, this phenomenon is illustrated by the overlap in fate between mesodermal derivatives and those of the ectodermally derived neural crest (reviewed in Gilbert, 1997). It is conceivable that nerve or muscle cells produced in different lineage contexts might not be strictly equivalent, differing subtly in the repertoire of genes they express. However, there seems to be no evidence to support such a view. Hence, what is the significance of cell lineage for cellular diversification? Given the evidence from cell fusion, nuclear transplantation and other studies that the differentiated state is maintained by continuous regulation of gene expression (Blau, 1989; Blau & Baltimore, 1991; Gardner, 1993), what is there to prevent one type of cell forming any other type when it is placed in an appropriate environment? Early in development, before different environments and stem cell niches are formed, it is vital that cells acquire stable differences that are independent of such features: otherwise cellular diversification could not occur. However, once tissues have differentiated and distinct niches for different types of stem cells have formed, the requirement for such stability would seem less obvious. Indeed, retention of plasticity in the differentiative potential of stem cells in adults, as seems to be the case even in human bone marrow chimaeras (Alison et al. 2000; Theise et al. 2000), might be advantageous.

Public perception

Early attempts to establish enduring cell cultures from early stages of development in mammals were made by Robert Edwards and colleagues who explanted rabbit and mouse pre-implantation stages onto various substrata, including extracellular matrices and irradiated feeder cells (Cole & Paul, 1965; Cole et al. 1965, 1966). The aim was to avail themselves of a 'large homogeneous population of embryonic cells in which the synthesis of a specialised protein could be initiated *in vitro* by a specific stimulus of developmental significance, and in which studies could be extended to cell-free extracts.' This was essentially the same purpose for which many adopted EC cells for *in vitro* study, first in the mouse and later in man. One of the attractions of EC cells was undoubtedly the ease with which they could be expanded indefinitely whilst retaining an undifferentiated appearance. By contrast, very few enduring cell lines were obtained from blastocyst explants, and all showed more or less obvious features of differentiation (Cole et al. 1966; Sherman, 1975). Nonetheless, it is interesting to speculate that, had EC cells not offered themselves as a convenient alternative to the blastocyst, the discovery of ES cells might conceivably have occurred as much as a decade earlier.

The idea of harnessing embryo-derived cells for therapeutic purposes seems, like many others, to have first been articulated by Robert Edwards. He made passing reference to it in a book charting the history of human *in vitro* fertilization (Edwards & Steptoe, 1980). Two years later, he was more explicit in dealing with two options that were open at that time (Edwards, 1982). The first was to obtain organized post-implantation development of human IVF conceptuses *in vitro* to a stage when organ primordia would be available for transplantation. The other, prompted both by his own much earlier work with Cole and Paul and by very recent success in obtaining ES cells from mice cells (Evans & Kaufman, 1981; Martin, 1981), was to derive cell lines from pre-implantation stages that could be made to undergo the desired differentiation *in vitro*. The first option was, of course, closed in the UK with passing of the Human Fertilization & Embryology Act in 1990.

Peter Hollands, one of Edwards' students, seems to have been the first to attempt to demonstrate the therapeutic potential of cells from early conceptuses in a laboratory as opposed to a clinical context. He reported functional colonization of the haematopoietic system of adult mice with cells from early post-implantation mouse conceptuses or even blastocysts, regardless of whether they had been lethally irradiated (Hollands, 1987, 1988a, 1988b). He also found that such grafts could cure genetically anaemic mice (Hollands, 1988c), and worked xenogeneically between rat and mouse (reviewed in Hollands, 1991). In initial experiments, the donor material consisted of entire early post-implantation stages within intact decidual tissue which is known to include cells originating from maternal bone marrow (Lysiak & Lala, 1992). However, early decidua appear to contain few such cells (Gambal et al. 1985), and subsequent experiments in which the donor material was from cultured pre-implantation stages, and therefore devoid of decidual tissue, yielded similar results (Hollands, 1988a). What remains very puzzling about these studies, and clearly exercised their author, was the extraordinary rate at which cells expressing donor markers, including that for globin, appeared in the blood. This seemed to demand a cycle time of the order of 1.5 h for which there is no precedent in mammals. Of further interest is that while cells from blastocysts grown *in vitro* exhibited such striking haematopoietic potential without any evidence of tumour formation, no colonization was discernible with similar numbers of established ES cells (Hollands, 1988a). It would be interesting to know more about the nature and status of the effective cells and, in the light of recent developments, whether their contribution was confined to the haematopoietic system or extended to other tissues of the adult hosts. Regardless, the idea of exploiting embryonic stem therapeutically is clearly not a new one, and the first steps towards its practical development occurred more than a decade before it gained wide currency within the biomedical research community.

There seems to be a general public perception that the pace of scientific advance is too unremittingly fast to allow proper consideration of the ethical and other broader issues relating to specific developments. In the case of human *in vitro* fertilization, this does not ring true. What was evident was a reluctance on the part of society to confront the issues that the technology raised until, with the birth of Louise Brown, it became clear that they could no longer be ignored. Thus, in the UK an interval of 21 years elapsed between the first demonstration of successful *in vitro* fertilization in man, when its future was clearly mapped out, and the introduction of relevant legislation (Table 1). Moreover, issues raised by stem cell therapy, such as the status of

Table 1 Chronology of development of human IVF in the UK

1969	IVF first achieved by Edwards, Bavister & Steptoe
1978	Birth of first IVF baby – Louise Brown
1982	HM Government set up Warnock Committee
1984	Warnock Committee reports to Government
1990	Human Fertilization and Embryology Act

Table 2 Chronology of development of embryonic stem cell therapy in the UK

1982	Concept of using cells from early human conceptuses therapeutically made explicit.
1987	Therapeutic potential of cells from early conceptuses demonstrated in a laboratory context.
1998	Human Genetics Advisory Committee and Human Fertilization and Embryology Authority issued consultation paper entitled: *Cloning issues in reproduction, science and medicine*.
HM Government responded by asking Chief Medical Officer to set up an Expert Group to look at issue of therapeutic cloning.	
2000	The CMO's Expert Group recommended that therapeutic cloning, and hence stem cell research, should be permitted.
2000	Human Fertilization and Embryology (Research Purposes) Regulations 2000 – approved by the House of Commons.
2001	Human Fertilization and Embryology (Research Purposes) Regulations 2000 – approved by the House of Lords.

preimplantation stages of development, were rehearsed extensively during this earlier period of very protracted debate. However, regarding stem cell therapy, the public perception is probably more accurate because the wider debate could not begin until the scientific community was persuaded of its merits and this, as indicated above, took a surprisingly long time. Moreover, the start of the wider debate seems to have owed more to the birth of Dolly the sheep than to any scientific advocacy of the clinical potential of ES cells (Table 2).

Finally, there are two other points that colour the public perception of work in this field. The first stems from the cavalier way in which mammalian embryologists employ the term embryo, by applying it to any stage of development beyond the zygote. This presents problems in communication even among workers in the field when, for example, it is necessary to differentiate between axes that relate to the entire entity vs. the fetus itself (Gardner, 2001). Such qualifying terms as 'embryo proper' or 'nascent fetus' have to be used to distinguish the latter from the former. Once the public debate on use of human pre-implantation stages of development for research began, it was recognized that describing these stages as embryos was liable to convey the false impression that they were essentially fetuses in miniature. Hence, the terms 'pre-embryo' or 'pro-embryo' were introduced, and understandably engendered suspicion that they were simply euphemisms. Surprisingly, the time-honoured term 'conceptus', embracing all the products of conception, was not adopted by mammalian developmental biologists at that juncture. However, in the interest of eliminating ambiguity in communicating with each other as well as more widely, there seems to be a strong case for adopting it now.

The second point that, with hindsight, seems to have been unhelpful, is the coupling of so-called 'therapeutic cloning' with stem cell therapy as if the two were strictly interdependent. As noted earlier, it was the issue of therapeutic cloning rather than ES cells *per se* that started the public debate in the UK. The case for vigorously exploring the potential of ES cells derived from conceptuses that are surplus to requirements of assisted reproduction has now been endorsed almost universally within the biomedical community. The question of whether therapeutic cloning will ever amount to more than a research tool for aiding a better understanding of reprogramming of gene expression remains more contentious. It is conceivable that direct re-specification of cells of patients to whatever type they require will, in the end, prove a more realistic option. The derivation of ES cell lines for therapeutic purposes does not seem to pose any novel ethical issues, especially since use of tissue from aborted fetuses for such purposes was sanctioned some years ago in the UK. Public concern is focused more on the issue of where therapeutic cloning, which may not even prove to be a viable option therapeutically, might lead.

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